Research Article

Received: 9 May 2008 Revised: 9 September 2008 Accepted: 10 September 2008 Published online in Wiley Interscience: 29 December 2008

(www.interscience.wiley.com) DOI 10.1002/ps.1687

Influence of glyphosate, crop volunteer and root pathogens on glyphosate-resistant wheat under controlled environmental conditions

George J Baley,[a] Kimberley G Campbell,[b] Joseph Yenish,[a] Kimberlee K Kidwell[a] and Timothy C Paulitz[c]*

Abstract

BACKGROUND: The herbicide glyphosate has a synergistic effect on root disease because of increased susceptibility from reduced plant defenses resulting from the blockage of the shikimic acid pathway. Could glyphosate-resistant (GR) wheat cultivars and glyphosate application in-crop increase the risk of damage from soil-borne pathogens? Growth chamber experiments were conducted with two GR wheat lines and their corresponding glyphosate-sensitive (GS) parents and four pathogens (*Rhizoctonia solani* Kühn *R. oryzae* Ryker & Gooch, *Gaeumannomyces graminis* (Sacc.) v. Arx & J. Olivier var. *tritici* J. Walker and *Pythium ultimum* Trow). Treatments consisted of different herbicide timings and planting of crop volunteer to mimic management practices in the field.

RESULTS: GR cultivars were not inherently more susceptible to root pathogens than GS cultivars, and application of glyphosate did not increase root disease. When crop volunteer was grown in close proximity to GR cultivars, the timing of glyphosate application had a profound effect. In general, the longer the crop volunteer was left before killing with glyphosate, the greater was the competitive effect on the planted crop. Both *R. solani* and *G. graminis* var. *tritici* reduced plant height, number of tillers and root length of the GR cultivars in the presence of crop volunteer with glyphosate application.

CONCLUSION: To minimize the damaging effects of these pathogens, producers should apply glyphosate at least 2–3 weeks before planting GR wheat, as currently advised for GS cereals.
Published 2008 by John Wiley & Sons, Ltd.

Keywords: glyphosate resistance; wheat; *Rhizoctonia solani*; *Rhizoctonia oryzae*; *Gaeumannomyces graminis*; *Pythium ultimum*; greenbridge effect

1 INTRODUCTION

Weed control, especially the control of grassy weeds in no-till wheat (*Triticum aestivum* L.) production is problematic owing to the lack of cultivation as a weed management tool. Herbicides are a primary input cost in wheat production systems in the Pacific Northwest (PNW) of the USA, and the declining price of glyphosate and the increasing price of fuel are shifting the economics in favor of no-till compared with conventional wheat production.[1] The most commonly used herbicides in cereal grain production are limited to just a few modes of action, which has resulted in widespread development of herbicide-resistant weed populations. In cereals, group-1 and group-2 herbicide-resistant weed populations are the most common. In the PNW, group-1 resistant populations of wild oats and Italian ryegrass have been reported in wheat production systems, while group-2 resistant populations of Italian ryegrass, kochia, prickly lettuce, Russian thistle and spiny sowthistle have also been reported (International Survey of Herbicide Resistant Weeds, Weed Science Society of America; www.weedscience.org). Transgenic glyphosate-resistant (GR) wheat cultivars could provide an efficient tool for weed control in no-till cropping systems. Glyphosate, a non-selective post-emergence herbicide, does not persist in the environment,[2] and is widely used in preplant applications for wheat production. However, glyphosate has a unique interaction with root pathogens. Glyphosate inhibits the enzymatic activity of 5-enolpyruvoyl shikimate 3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway.[3,4] This pathway is needed for the formation of aromatic amino acids, key components of plant defense pathways, especially for the formation of phenolic compounds.[5–7] The alternative form of EPSPS (*CP4* EPSPS) expressed by GR wheat is not affected by glyphosate;[8] however, the disease response of GR wheat after treatment with glyphosate remains unknown. When glyphosate-sensitive

* *Correspondence to: Timothy C Paulitz, USDA-ARS, Root Disease and Biological Control Unit, Pullman, WA 99164-6430, USA. E-mail: paulitz@wsu.edu*

a *Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA*

b *USDA-ARS, Wheat Genetics, Quality, Physiology, and Disease Research Unit, Pullman, WA 99164-6420, USA*

c *USDA-ARS, Root Disease and Biological Control Unit, Pullman, WA 99164-6430, USA*

(GS) plants are treated with glyphosate, root diseases caused by soil-borne pathogens can increase because of the disabled defense pathways. This is known as the 'glyphosate synergistic response' and has been documented with *Pythium* spp.[9–11] To exacerbate this situation further, dying weeds or volunteer crop can serve as a source of inoculum for cereal crops planted soon after glyphosate application. The transmission of inoculum and pathogen from dying weeds and crop volunteers to the subsequent crop is known as the 'greenbridge effect' and has been widely documented with *Rhizoctonia* and other diseases, such as take-all (caused by *Gaeumannomyces graminis* var. *tritici*) and Pythium root rot (caused by numerous *Pythium* spp.).[10,12,13] In addition, pathogens that show a 'greenbridge effect' are often a greater problem in no-till compared with conventional tillage systems.[14–17] Tillage breaks apart hyphal networks in the soil, accelerates the decomposition of crop residues that contain pathogen inoculum and results in an increase in microbial activity that may inhibit pathogens. There is no genetic resistance to these pathogens in adapted cultivars,[18–21] although some cultivars are more tolerant than others,[22,23] and genetic tolerance to *Rhizoctonia* has been identified in a mutagenized spring wheat line (Okubara PA *et al*., unpublished). The current best management recommendation for growers is to apply herbicide to weeds at least 3 weeks prior to planting to allow pathogen inoculum to decline as a result of microbial activity.[24] However, with the advent of GR wheat, GS weeds or volunteer GS crop can be killed with herbicide within the growing crop, and these dying plants could serve as a reservoir of inoculum that infects the GR crop. Hence, the 'greenbridge' effect may be exacerbated in GR wheat production.

Several public universities and private seed companies collaborated with the Monsanto Corporation (St Louis, MO) from 1999 to 2004 to develop adapted (GR) spring wheat cultivars for each major production region in the United States.[8] Numerous GR crops, including canola, corn, cotton and soybean, have been available for commercial production since 1996.[25–27] In 2006, 89% of the soybean, 21% of the corn and 26% of the cotton in commercial production in the USA was herbicide resistant.[28] GR wheat was expected to be well received by the agriculture community, based on its potential benefits, which include increased crop safety, improved environmental stewardship and enhanced control of weed biotypes resistant to other herbicides.[29] Monsanto discontinued the development of GR wheat in 2004. Nevertheless, transgenic wheat cultivars may be released in the future, especially if cultivars express herbicide resistance along with consumer-valued traits, which may be more acceptable to global markets.[30,31] Prior to the commercial release of GR wheat cultivars, the risk of increased root disease pressure resulting from the greenbridge effect should be proactively assessed. Data on the interactions between glyphosate–weed management systems and soil-borne root pathogen levels in GR crop species are limited, and nothing is known about these relationships in GR wheat crops.

The present authors designed growth chamber experiments with pathogen inoculation to answer the following questions:

1. Are GR wheat cultivars inherently more susceptible to root pathogens than GS wheat cultivars?
2. Does the application of glyphosate to GR wheat cultivars increase their susceptibility to root pathogens?
3. Does the presence of volunteer crop in close proximity to GR wheat cultivars increase root disease?
4. Does the timing of application of glyphosate in the presence of volunteer affect root disease?

This research focused on four fungal root pathogens: *Pythium ultimum* Trow, *Gauemannomyces graminis* (Sacc.) v. Arx & J. Olivier var. *tritici* J. Walker (*Ggt*), *Rhizoctonia solani* Kühn AG-8 and *R. oryzae* Ryker and Gooch. Field trials with GR and GS wheat were performed at three locations in inoculated field trials, but it was difficult to establish high pathogen levels in these trials.[32] With growth chamber experiments, it was possible to limit environmental interactions and apply uniform pathogen inoculum in order for these questions to be more accurately addressed.

2 MATERIALS AND METHODS

2.1 Effect of glyphosate and soil-borne pathogens on GR and GS wheat, in the absence of volunteer crop

2.1.1 *Inoculum and soil preparation*

All pathogens evaluated in this study were collected, isolated and maintained by the USDA-ARS Root Disease and Biological Control Research Unit at Pullman, WA. Inoculum of *G. graminis* var. *tritici* (isolate R3-111a-1), *R. solani* (AG-8 isolate C1) and *R. oryzae* (isolate 801 387) was produced on oat (*Avena sativa* L.) seed autoclaved in 1000 mL wide-mouth Erlenmeyer flasks.[18] Inoculum of *P. ultimum* (isolate 010 143) was produced as described by Higginbotham *et al*.[22] Before infesting the soil, *P. ultimum* inoculum was serially diluted and placed on *Pythium*-selective media (PSM) to determine the inoculum density.[33]

Thatuna silt loam soil, obtained from Spillman Farm (Washington State University, Pullman, WA) was placed in a steam cart (Siebring Mfg Inc., George, IA) and pasteurized at 60 °C for 30 min. *Gauemannomyces graminis* var. *tritici*-, *R. solani*- and *R. oryzae*-colonized oats were ground into a course meal using a coffee grinder and added to 3900 g of preweighed soil in individual plastic bags at rates of 0.175% (w/w) for *R. solani* (AG-8) and *R. oryzae* (801 387) and 0.20% for *G. graminis* var. *tritici*. Pasteurized soil for *P. ultimum* treatments was infested at a population density of 500 colony-forming units (CFUs) g^{-1} and amended with 0.15% (w/w) ground rolled oats as a food base to increase inoculum potential.[22,34] Infested soils were manually agitated to ensure thorough distribution of inoculum, and dispensed into plastic 'cone-tainers'™ (4 cm diameter × 20.5 cm long; Ray Leach 'Cone-tainer'™, Canby, OR) at 130 g soil $tube^{-1}$. Non-inoculated controls were prepared similarly to infested soils, with the omission of oatmeal or pathogen amendments.

2.1.2 *Genotype descriptions*

Four hard red spring wheat genotypes consisting of two sets of near-isogenic lines (NILs) differing in resistance to the herbicide glyphosate were evaluated under controlled environment conditions. The GS genotypes evaluated were GS Bobwhite (CM33203; CIMMYT, Mexico) and GS Westbred 926 (WestBred LCC, Bozeman, Montana). GS Bobwhite is not adapted to the PNW, but was included in this study since it was the initial wheat genotype transformed with the glyphosate resistance construct *CP4* ESPS.[8,35] In contrast, GS Westbred 926 is a commercial cultivar widely grown in the region. The GR genotypes evaluated were GR Bobwhite (event 33 391;[8] Monsanto Co, St Louis, MO] and GR Westbred 926 (WestBred LCC, Bozeman, Montana). Following regeneration, GR Bobwhite transgenic plants were self-pollinated for five generations, and resulting R_6 generation material was evaluated in this study. GR Bobwhite was originally distributed by the Monsanto Co. for utilization as the donor parent for *CP4* ESPS gene introgression into adapted wheat genotypes.[8] GR

Westbred 926 was developed by cross-hybridizing GR Bobwhite and GS Westbred 926. Resulting F_1 plants were backcrossed for four generations to GS Westbred 926, followed by self-pollination for five generations to create BC_4F_6 GR Westbred 926, which was used in this study. By BC_4, the recurrent parent genome should represent 97% of the total genome.

2.1.3 Growth chamber conditions

Plastic 'Cone-tainers'™ were suspended in racks and placed in a 2.7 × 2.7 m Conviron growth chamber (GR48, Controller Environments Limited, Winnipeg, Canada) programmed for a 12 : 12 h light : dark photoperiod at a constant 16 °C and 70% relative humidity. The soil in each 'Cone-tainer'™ was watered with 10 mL of distilled water, as needed to prevent hardening of the soil surface and plant desiccation. All genotypes were pregerminated in petri dishes with moist filter paper for 48 h at room temperature prior to planting. Pregerminated seeds were placed on the soil surface and covered with 10 cm^3 of pasteurized Thatuna silt loam soil. Growth chamber space limitations required evaluations to be conducted in two separate experiments. Near-isogenic line (NIL) sets were evaluated with *Rhizoctonia* spp. (*R. oryzae* and *R. solani*) in experiment 1, and with *G. graminis* var. *tritici* and *P. ultimum* in experiment 2. Both experiments included non-inoculated controls to compare with pathogen treatments.

2.1.4 Glyphosate applications

Once seedlings reached the 3–4-leaf growth stage (Zadoks growth stage 1.3–1.4),[36] glyphosate applications were made in a contained spray booth (Research Instruments, Co., Guelph, ONT) calibrated to deliver 80 L ha^{-1} at 262 kPa using a single SS8001E nozzle tip (TeeJet, Wheaton, IL) from a height of 45 cm above the plant canopy. GR genotypes received either 0.2 or 0.8 kg glyphosate AE ha^{-1} (Roundup Ultra; 480 g L^{-1} in the form of its isopropylamine salt; Monsanto Co., St Louis, MO). The expected labeled rate of glyphosate for GR wheat is 0.8 kg AE ha^{-1},[8] whereas 0.2 kg AE ha^{-1} of glyphosate disrupts the shikimic acid pathway but does not cause plant death of GS wheat.[37] GS genotypes only received the sublethal 0.2 kg glyphosate AE ha^{-1} rate, since the higher field rate of glyphosate would prove lethal. An unsprayed (0.0 kg glyphosate AE ha^{-1}) control was included for each genotype.

2.1.5 Data collection and analysis

Seedlings were removed from 'Cone-tainers'™ 1 day before glyphosate treatment (−1 DAT), and at 7 days after (7 DAT) and 12 days after (12 DAT) treatment with glyphosate. Soil was gently removed from the roots using a high-pressure stream of tap water. Plant height, length of first emerged leaf and number of crown and seminal roots were recorded. The numbers of crown and seminal roots with visible symptoms of infection by *R. solani, R. oryzae* or *G. graminis* var. *tritici* were also recorded. Roots were removed from plants at the crown, placed in 20.5 × 20.5 × 1.5 cm glass trays with 200 mL distilled water and spread out manually to minimize overlapping. Remaining soil debris was removed by hand, and the image was captured with a digital scanner (Epson Expression 1680; Epson America Inc., Long Beach, CA). Digital root analyses (WinRhizo Pro Version 5.0a; Quebec, Canada) were used to measure total root length, average root diameter and the number of root tips.[38]

The experiment was designed as a split plot with five blocks. Glyphosate treatments were the main plot factor, and a factorial arrangement of genotype by pathogen treatments were the subplot factors.[39] Sensitivity of GS genotypes to 0.8 kg glyphosate AE ha^{-1} required nesting of wheat genotypes within glyphosate treatments. Data from the two experiments were analyzed separately through analysis of variance using the SAS PROC MIXED procedure (Version 8.0; SAS Institute Inc., Cary, NC). Block and block by glyphosate treatment were specified as random effects, whereas glyphosate treatments, genotypes, pathogens and their interactions were considered fixed. Multiple mean comparisons were accomplished by the Tukey–Kramer test at the 5% significance level.[40]

2.2 Effect of glyphosate and soil-borne pathogens on GR wheat in the presence of GS volunteer wheat (simulated greenbridge)

Inoculum and soil were prepared as described in Section 2.1. This experiment was conducted in larger plastic containers (D40 Deepot; 6.4 cm diameter × 25 cm long; Stuewe & Sons Inc., Corvallis, OR) filled with 350 g of infested or non-infested pasteurized soil. For treatments that included volunteer plants, three seeds of the winter wheat cultivar 'Eltan' were planted into containers to simulate volunteer wheat for greenbridge establishment. Growth chamber conditions were as described above.

2.2.1 Glyphosate–weed management systems

Six different weed management systems were established to control GS volunteer wheat (Fig. 1). Treatment 1 (PRE) simulated current glyphosate–weed management systems recommended for GS cereals in the Pacific Northwest. An initial single flush of GS volunteer wheat growing in containers was treated with glyphosate 2 weeks prior to planting GR wheat genotypes. Treatment 2 (PRE + POST) simulated a split application of glyphosate to GR wheat. An initial single flush of GS volunteer winter wheat was introduced into containers and treated with glyphosate 2 weeks prior to planting GR genotypes. A second flush of GS volunteer winter wheat was planted into containers at the same time as the GR genotypes and was controlled 'post-emergence' with glyphosate. This treatment mimics the most likely management scenario for GR wheat. Treatment 3 (PLANTING) simulated a weed management system in which glyphosate was applied to control GS volunteer winter wheat in containers the same day that GR genotypes were planted. Treatment 4 (POST) simulated a weed management system where a single glyphosate application was used to control GS volunteer cereals post-emergence of GR wheat genotypes. A single flush of volunteer winter wheat was planted at the same time as in treatments 1–3 described above; however, volunteer wheat was allowed to grow past the time of planting GR genotypes in the same container. Both GS volunteer winter wheat plants and GR genotypes were treated POST with glyphosate on the same date. Treatment 5 (NO-VOL) simulated a weed management system in which no volunteer GS winter wheat was present at the time of glyphosate application. Inoculated soil was prepared and maintained volunteer-free for the duration of the experiment. GR genotypes were planted and sprayed with glyphosate after emergence. Treatment 6 (NO-VOL/NO-SPRAY) was identical to treatment 5, except that glyphosate was not applied to GR genotypes at any time during the experiment. Treatments 5 and 6 were nearly identical to treatments in the experiment with no volunteers, described in Section 2.1.

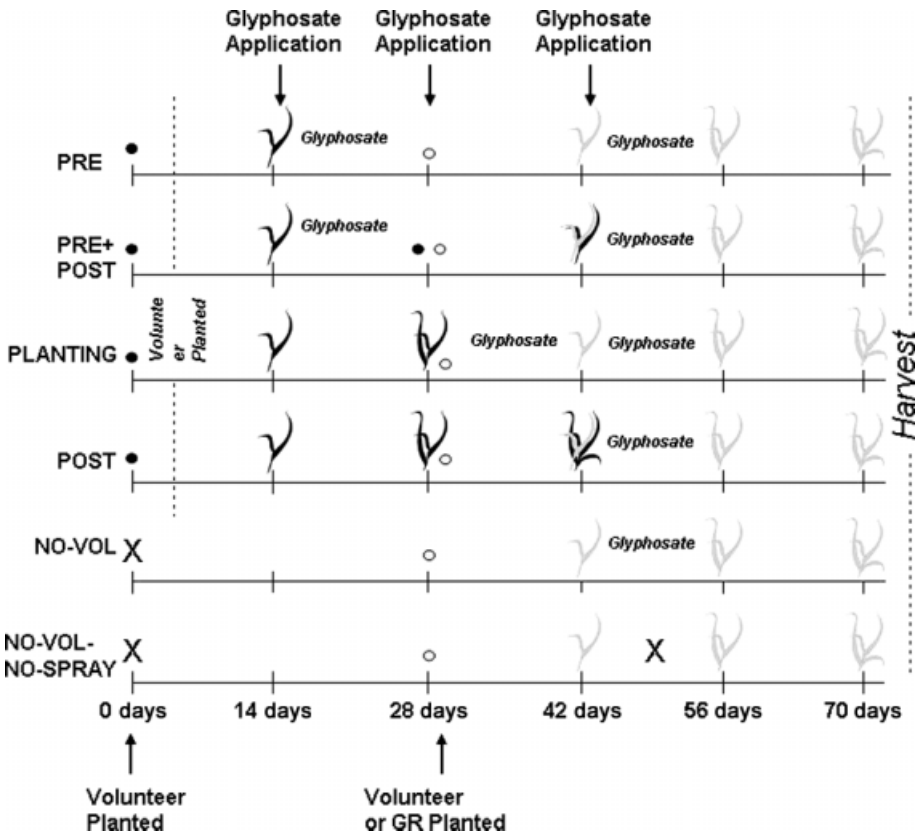

Figure 1. Timeline of the establishment of volunteer winter wheat and GR spring wheat genotypes and the timing of glyphosate applications: preplant (PRE), preplant and post-emergence (PRE + POST), planting (PLANTING), post-emergence (POST), no-volunteer (NO-VOL) and no-volunteer/no-spray (NO-VOL/NO SPRAY). All volunteer (cv. Eltan) seeds and plants are represented in solid black, and all GR spring wheat seeds and plants are represented in gray.

The experiment was designed as a split plot with five replicates. Glyphosate–weed control treatments were the main plots, and subplots consisted of a factorial arrangement of genotype by pathogen treatments. Each glyphosate–weed management system by pathogen treatment was replicated 5 times for each genotype, pathogen and management system. Racks of containers for each glyphosate–weed management systems were positioned in randomly assigned locations in the growth chamber.

All GR genotypes were treated with 0.8 kg glyphosate AE ha^{-1} (Roundup Ultra; 480 g L^{-1} in the form of its isopropylamine salt; Monsanto Co., St Louis, MO), with the exception of NO-VOL/NO-SPRAY glyphosate–weed management treatments. Herbicide applications were made as described in Section 2.1.

2.2.2 *Pathogen quantification*

Quantification of *R. solani, R. oryzae* and *P. ultimum* activity within infested soils was measured at 7-day intervals from the initial glyphosate application to the first flush of volunteer winter wheat until the end of the experiment for PRE + POST, PLANTING and NO-VOL/NO-SPRAY treatments. Weed management strategies for PRE, POST and NO-VOL treatments were identical to PRE + POST, PLANTING and NO-VOL/NO-SPRAY respectively until GR genotypes were planted (Fig. 1). For this reason, soil-borne pathogen activity levels for PRE, POST and NO-VOL treatments were only quantified from the time GR genotypes were planted until the end of the experiment. *Gauemannomyces graminis* var. *tritici* was not quantified owing to the lack of a suitable, non-destructive measurement technique.

The hyphal activities of *R. solani* and *R. oryzae* were measured using a modified toothpick colonization technique.[41] Five toothpicks (5.5 cm long) were randomly inserted into the soil of five randomly selected individual containers infested with *R. solani* or *R. oryzae* for each glyphosate–weed treatment. Once a container was selected for quantification of *Rhizoctonia* spp., that container was repeatedly sampled at approximately 7-day intervals. The ends of toothpicks (0.5 cm) were left exposed above the soil surface to facilitate removal with forceps after 48 h. Excess soil was shaken off, and all five toothpicks per container per sampling time for each treatment were placed on a 90-mm petri plate containing *Rhizoctonia*-selective medium. This medium consisted of water agar amended with 1 mg L^{-1} benomyl to inhibit non-target fungal growth and 100 mg L^{-1} chloramphenicol to inhibit bacteria. After 24 h, the numbers of colonies growing from each toothpick were counted using a dissecting microscope (Olympus; Tokyo, Japan) using a grid of 5-mm squares placed under the plate. Three containers each from non-inoculated treatments and those infested with *P. ultimum* or *G. graminis* var. *tritici* were randomly selected and sampled with toothpicks on the same dates that *Rhizoctonia* spp. were sampled to ensure that contamination had not occurred.

Population densities in *P. ultimum*-infested soils were quantified by sampling 3 g of soil 3 cm below the soil surface for five randomly selected *P. ultimum*-infested containers for each weed control treatment. Once a container was randomly selected for *P. ultimum* quantification, repeated sampling occurred throughout the experiment at approximately 7-day intervals. Soil samples were diluted by adding 1 g of soil to 9 mL of distilled water and

vortexing for 30 s, resulting in a 10^{-1} dilution. One mL of the soil suspension was added to 9 mL of distilled water to create a 10^{-2} dilution. A 0.5 mL aliquot of the 10^{-2} dilution was placed onto *Pythium*-selective medium[33] in 90-mm petri plates and spread with a sterile glass rod. Plates containing diluted soil suspensions were stored in the dark, and colonies were counted after 24 h using a dissecting microscope (Olympus; Tokyo, Japan). Soil samples from non-inoculated treatments and from those infested with *R. solani, R. oryzae* or *G. graminis* var. *tritici* treatments also were collected on the same sampling dates to ensure that contamination of soils with *P. ultimum* had not occurred.

2.2.3 *Data collection and analysis*

Data were collected at the end of the experiment (70 days after planting), as described in Section 2.1. Data collected for plant health parameters and *R. solani* activity were subjected to analysis of variance using the SAS mixed model procedure to obtain least-square means (Version 8.0; SAS Institute Inc., Cary, NC). Block main effects and interactions were treated as random effects, whereas glyphosate–weed management systems, genotypes, pathogens and their interactions were considered fixed for the randomized complete block split-plot design. Mean comparisons for plant health parameters were accomplished by the Tukey–Kramer test at the 5% significance level, and standard errors were generated for *R. solani* activity for each glyphosate–weed management treatment for each sampling date.

3 RESULTS

3.1 Effect of glyphosate and soil-borne pathogens on GR and GS wheat, in the absence of volunteer crop

Of the traits measured, only plant height and total root length are presented here. Other variables showed similar trends compared with reported traits; however, the reported variables were the most responsive to pathogen and herbicide treatments. In addition, only the data from 12 DAT are presented.

In the experiment with the two *Rhizoctonia* spp., the pathogen had a significant effect ($P \leq 0.001$) on both plant parameters, both before and after treatment with glyphosate. *R. solani*, but not *R. oryzae*, significantly reduced both plant height and root length in all genotype/glyphosate treatments, compared with non-inoculated controls (Figs 2 and 3). No significant glyphosate by pathogen interactions were detected ($P = 0.97$ and 0.41 for height and root length respectively), indicating that the reduction in plant parameters caused by *R. solani* was similar across glyphosate treatments. In other words, GS wheat genotypes were as susceptible to *R. solani* as GR genotypes across glyphosate treatments. Glyphosate treatments alone did not have a significant main effect ($P = 0.22$ and 0.35 for height and root length respectively). In other words, application of full doses of glyphosate to GR cultivars and sublethal doses of glyphosate to the GS cultivars did not reduce plant height or root length. However, because of the severe effect of *R. solani* on root length, additional reductions due to glyphosate would not be possible to detect.

In the experiment with *G. graminis* var. *tritici* and *Pythium ultimum*, pathogen had a significant effect on both plant height and root length ($P \leq 0.001$), but not glyphosate treatment ($P = 0.31$ and 0.25 respectively). Nearly all *G. graminis* var. *tritici*-inoculated GS and GR genotypes demonstrated significant ($P \leq 0.05$) decreases in plant height and total root length compared with non-inoculated genotypes (Figs 4 and 5). In contrast, *P. ultimum* did not reduce plant parameters in treatments without glyphosate. However, the pathogen by glyphosate interactions due to *Pythium* were nearly significant ($P = 0.06$) and highly significant ($P = 0.007$) for plant height and root length respectively. Glyphosate treatment interacted with *P. ultimum* to cause significant ($P \leq 0.05$) damage to GS Bobwhite treated with 0.2 kg ha^{-1} glyphosate, compared with *P. ultimum*-inoculated GS Bobwhite without glyphosate application and *P. ultimum*-inoculated GR Bobwhite treated with either rate of glyphosate. In contrast, *P. ultimum* did not reduce plant parameters of GR Bobwhite or Westbred 926, either without or with glyphosate application.

3.2 Effect of glyphosate and soil-borne pathogens on GR wheat in the presence of GS volunteer wheat (simulated greenbridge)

3.2.1 *Quantification of* Rhizoctonia solani *activity*

Initial (week 3) levels of *R. solani* activity were similar among glyphosate–weed management systems (Fig. 6). By week 5, *R.*

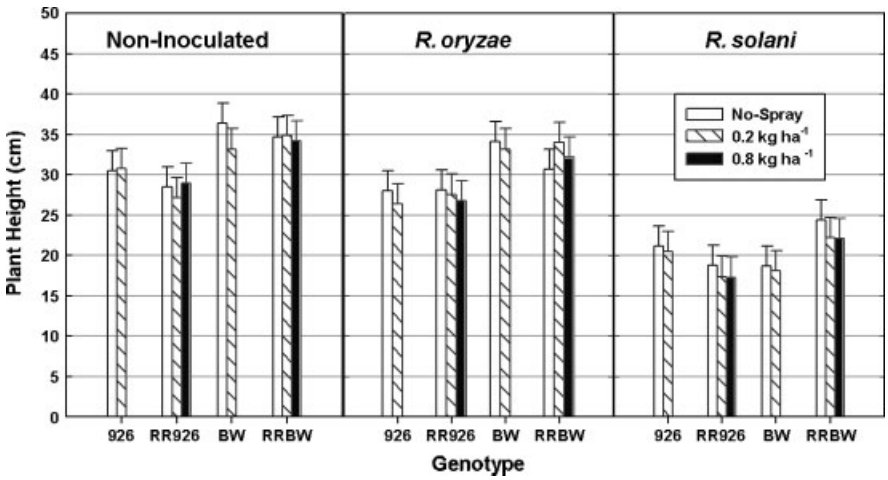

Figure 2. Plant height of glyphosate-resistant Bobwhite (RRBW) and 926 (RR926) and glyphosate-sensitive Bobwhite (BW) and 926 (926) inoculated with *Rhizoctonia solani* or *R. oryzae* and treated with 0.2 g glyphosate a.i. ha^{-1} or 0.8 g glyphosate a.i. ha^{-1} or no-spray control, evaluated 12 days after glyphosate treatment. Statistical significance bars represent significant differences ($P \leq 0.05$), Tukey's HSD test.

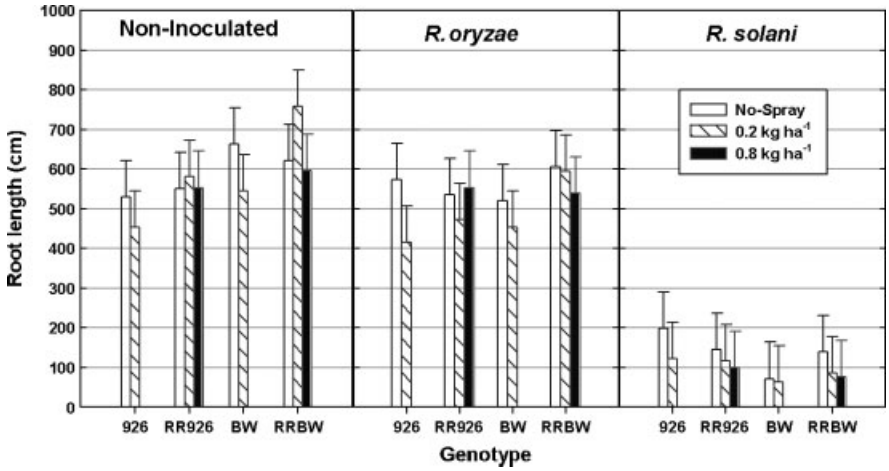

Figure 3. Root length of glyphosate-resistant Bobwhite (RRBW) and 926 (RR926) and glyphosate-sensitive Bobwhite (BW) and 926 (926) inoculated with *Rhizoctonia solani* or *R. oryzae* and treated with 0.2 g glyphosate a.i. ha^{-1} or 0.8 g glyphosate a.i. ha^{-1} or no-spray control, evaluated 12 days after glyphosate treatment. Statistical significance bars represent significant differences ($P \leq 0.05$), Tukey's HSD test.

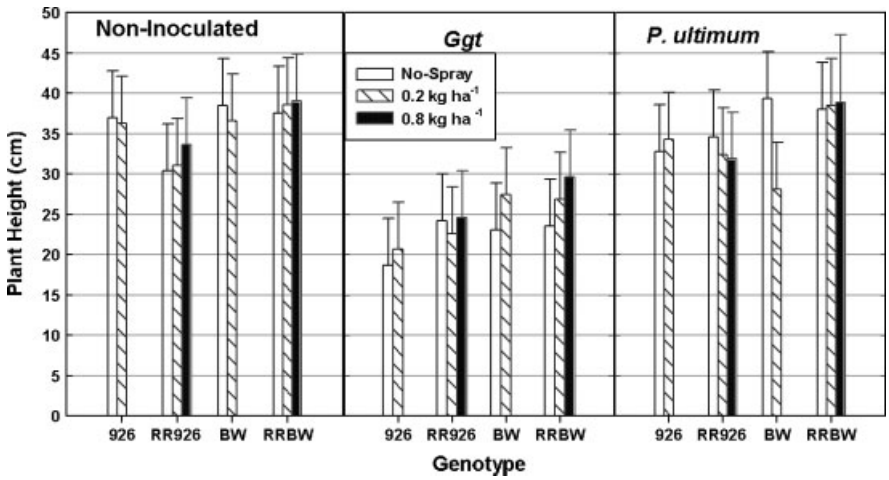

Figure 4. Plant height of glyphosate-resistant Bobwhite (RRBW) and 926 (RR926) and glyphosate-sensitive Bobwhite (BW) and 926 (926) inoculated with *Gauemannomyces graminis* var. *tritici* (*Ggt*) or *Pythium ultimum* and treated with 0.2 g glyphosate a.i. ha^{-1} or 0.8 g glyphosate a.i. ha^{-1} or no-spray control, evaluated 12 days after glyphosate treatment. Statistical significance bars represent significant differences ($P \leq 0.05$), Tukey's HSD test.

solani activity levels increased in containers with GS volunteer winter wheat. After week 5, *R. solani* activity levels significantly ($P \leq 0.05$) increased in soils from containers with growing GS volunteer winter wheat present at the time of glyphosate treatment. *Rhizoctonia solani* activity was highest in PLANTING and POST treatments from weeks 8 to 10, after glyphosate was applied to control GS volunteer winter wheat.

3.2.2 *Quantification of* Rhizoctonia oryzae *activity*

Colonies of *R. oryzae* were not detected using the toothpick method during these experiments.

3.2.3 *Quantification of* Pythium ultimum *activity*

The number of colony-forming units (CFUs) in *P. ultimum*-infested containers ranged from 3000 to 10 000 CFUs, which greatly exceeded the levels (500 CFUs) expected in nature (data not shown). Significant ($P \leq 0.05$) differences in *P. ultimum* CFUs among glyphosate–weed management systems were detected; however, CFUs remained relatively constant within management systems over sampling dates.

3.2.4 *Plant health assessments*

Of the plant health parameters measured, only plant height (Fig. 7), tiller number (Fig. 8) and total root length (Fig. 9) are presented here. Other parameters showed similar trends; however, the reported parameters were most responsive to glyphosate–weed management treatment. Plant heights, tiller numbers and total root lengths of genotypes significantly ($P \leq 0.0001$) varied among glyphosate–weed management systems and were significantly influenced by pathogen inoculation ($P < 0.001$). Significant ($P \leq 0.0001$) interactions were evident for glyphosate–weed management system by pathogen treatments. However, pathogen did not interact with genotypes ($P = 0.47$, 0.46 and 0.66 for plant height, number of tillers and root length respectively), so data were pooled across the two genotypes.

Both *G. graminis* var. *tritici* and *R. solani* decreased plant height in the PRE, PRE + POST and PLANTING treatments, but *R. oryzae* and *P. ultimum* showed no effect in these same treatments (Fig. 7). Interestingly, treatments with *P. ultimum* had greater plant heights than other treatments, but only in the POST herbicide treatment. In treatments without volunteer, pathogen inoculation

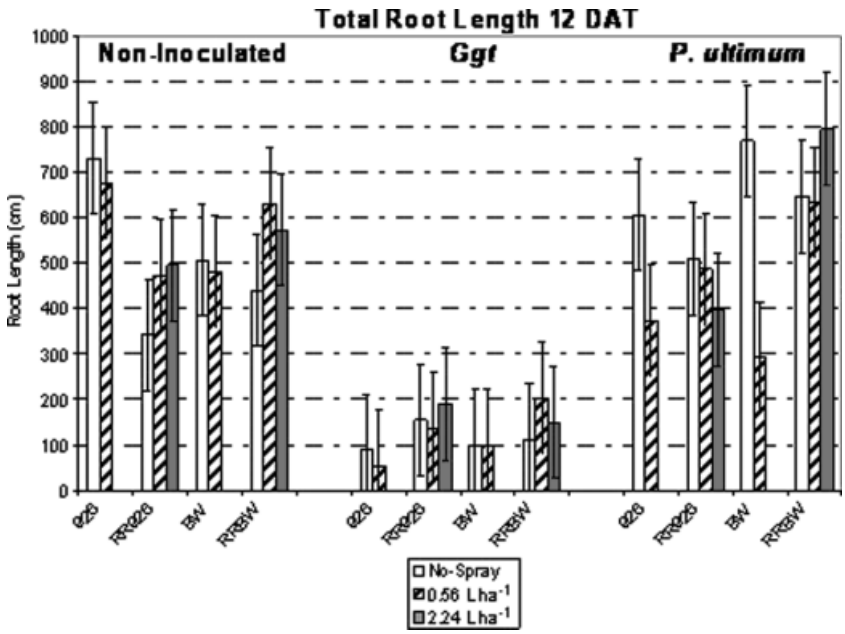

Figure 5. Root length of glyphosate-resistant Bobwhite (RRBW) and 926 (RR926) and glyphosate-sensitive Bobwhite (BW) and 926 (926) inoculated with *Gauemannomyces graminis* var. *tritici* (*Ggt*) or *Pythium ultimum* and treated with 0.56 L ha^{-1} Roundup Ultra or 2.24 L ha^{-1} Roundup Ultra, or no-spray control, evaluated 12 days after glyphosate treatment. Statistical significance bars represent significant differences ($P \leq 0.05$), Tukey's HSD test.

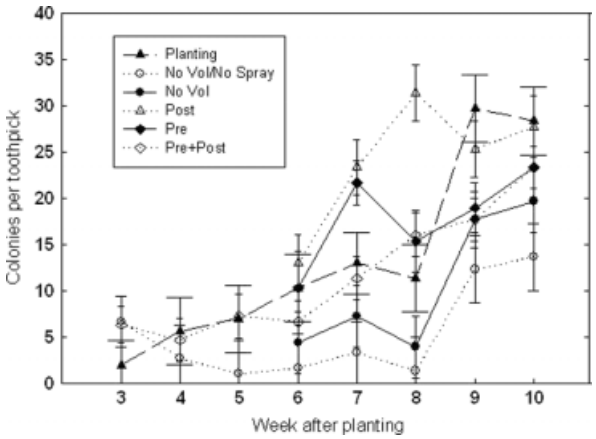

Figure 6. Quantification (colonies per toothpick) of *Rhizoctonia solani*-inoculated soil sampled from containers containing GR spring wheat genotypes untreated and treated with glyphosate in the presence and absence of GS winter wheat cv. Eltan for various weed management systems. Standard error bars signify differences ($P \leq 0.05$) between treatment means for each sampling date.

had no effect on plant height. A similar trend was observed on tiller number (Fig. 8). Both *G. graminis* var. *tritici* and *R. solani* inhibited tiller formation in the PRE and PRE + POST treatment. In the PLANTING and POST treatment, tiller formation was almost completely inhibited because of intense competition with the planted crop volunteer. However, this competitive inhibition of tiller formation was reduced with *P. ultimum* in the PLANTING treatment. Tillers were not inhibited by pathogens in treatments without volunteer. Root length showed similar trends to tiller numbers (Fig. 9). In general, *G. graminis* var. *tritici* and *R. solani* produced greater relative reductions in tiller number and root length compared with reductions in plant height. When control of volunteer plants was delayed, there was a general trend towards reduced plant parameters, with the POST treatment resulting in the most restricted plant growth. Results in the no-volunteer treatments were similar to those seen in the previous trial without volunteers (Section 3.1). *Rhizoctonia oryzae* and *P. ultimum* did not reduce plant parameters in the absence of volunteers. The disease response of both GR varieties was not affected by glyphosate application. In the first trial, both *R. solani* and G. *graminis* var. *tritici* reduced plant growth without volunteers.

4 DISCUSSION

A number of studies have documented the response of glyphosate-resistant soybean, cotton and sugar beet, but the disease responses of glyphosate-resistant wheat have not been previously reported. Treatment of glyphosate-sensitive plants with glyphosate can increase susceptibility to root pathogens. This phenomenon, known as 'glyphosate synergistic interaction', has been widely reported for *Pythium* spp.[10,42,43] Lévesque and coworkers[9,43] reported that *Pythium* spp. are primary root colonizers of glyphosate-treated plants; however, in the absence of foliar glyphosate applications, *Pythium* spp. rarely have been found within the root systems of mature plants.[44] Similarly, Lévesque *et al.*[11] reported increases in *Pythium* spp. within the rhizosphere as quickly as 2 h after the foliar application of glyphosate. In the present research, it was documented that treating glyphosate-sensitive Bobwhite with sublethal doses of glyphosate resulted in reduced plant height and root length, but only when inoculated with *P. ultimum*. This phenomenon of increased disease in the presence of glyphosate has been exploited to increase the efficiency of mycoherbicides, fungi applied to control weeds.[45,46]

One critical question is whether glyphosate-resistant plants have increased susceptibility to root pathogens, either in the presence or absence of glyphosate. If the glyphosate-sensitive parent had resistance to a pathogen, presumably this resistance would be present in the glyphosate-resistant transgenic crop. Genetic

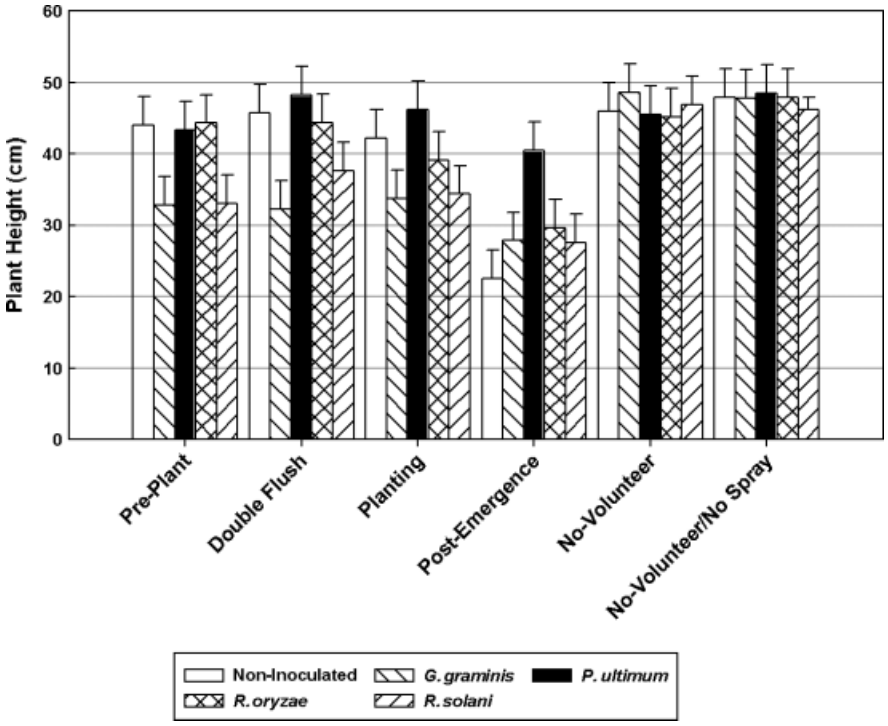

Figure 7. Mean plant height (cm) of GR wheat genotypes, non-inoculated and inoculated with *Gaeumannomyces graminis* var. *tritici, Pythium ultimum, Rhizoctonia oryzae* or *Rhizoctonia solani* in the presence and absence of volunteer winter wheat planted and treated with glyphosate. Six different glyphosate–weed management systems were evaluated: (a) preplant (PRE); (b) preplant and post-emergence (PRE + POST); (c) planting (PLANTING); (d) post-emergence (POST); (e) no-volunteer (NO-VOL); (f) no-volunteer/no-spray (NO-VOL/NO-SPRAY). Non-overlapping statistical significance bars signify significant differences by Tukey–Kramer multiple mean comparisons ($P \leq 0.05$).

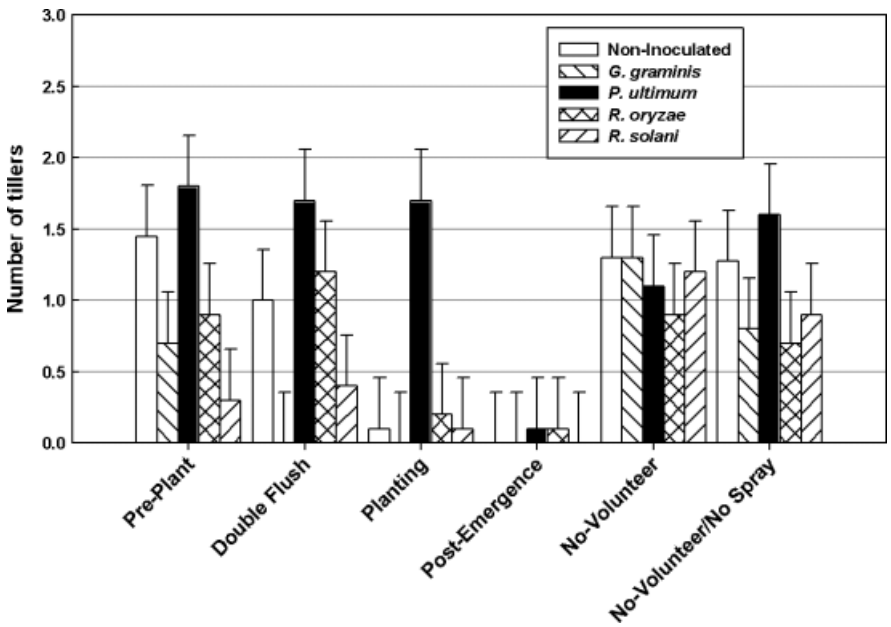

Figure 8. Mean tiller number produced by GR wheat genotypes non-inoculated and inoculated with *Gaeumannomyces graminis* var. *tritici, Pythium ultimum, Rhizoctonia oryzae* or *Rhizoctonia solani* in the presence and absence of volunteer winter wheat planted and treated with glyphosate. Six different glyphosate–weed management systems were evaluated: (a) preplant (PRE); (b) preplant and post-emergence (PRE + POST); (c) planting (PLANTING); (d) post-emergence (POST); (e) no-volunteer (NO-VOL); (f) no-volunteer/no-spray (NO-VOL/NO-SPRAY). Non-overlapping statistical significance bars signify significant differences by Tukey–Kramer multiple mean comparisons ($P \leq 0.05$).

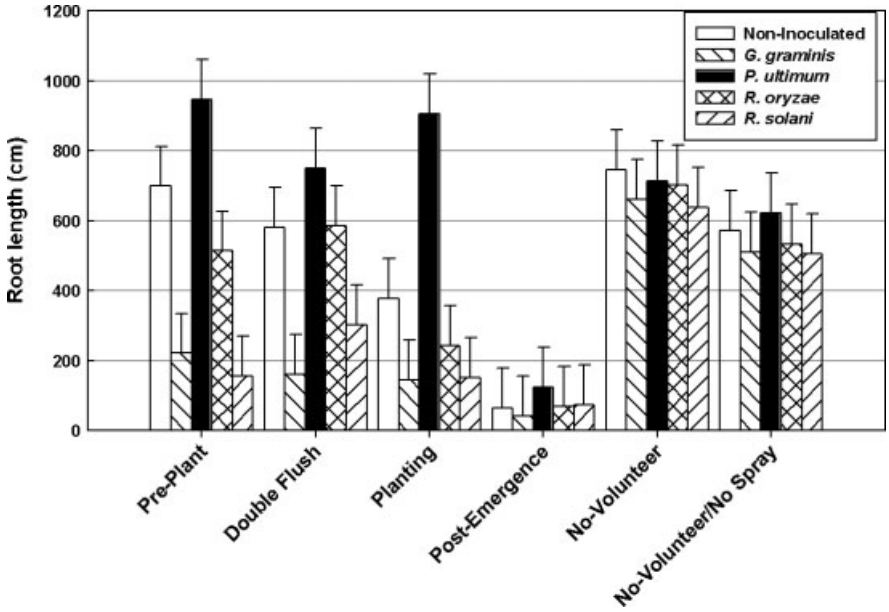

Figure 9. Mean root length (cm) of GR wheat genotypes non-inoculated and inoculated with *Gaeumannomyces graminis* var. *tritici, Pythium ultimum, Rhizoctonia oryzae* or *Rhizoctonia solani* in the presence and absence of volunteer winter wheat planted and treated with glyphosate. Six different glyphosate–weed management systems were evaluated: (a) preplant (PRE); (b) preplant and post-emergence (PRE + POST); (c) planting (PLANTING); (d) post-emergence (POST); (e) no-volunteer (NO-VOL); (f) no-volunteer/no-spray (NO-VOL/NO-SPRAY). Non-overlapping statistical significance bars signify significant differences by Tukey–Kramer multiple mean comparisons ($P \leq 0.05$).

resistance in wheat is not available for *Rhizoctonia solani*,[18,19] *G. graminis* var. *tritici* or *Pythium* spp.[23] The present authors did not see any increased susceptibility in the absence of glyphosate.

Do glyphosate-resistant plants show glyphosate synergism (i.e. increased disease with glyphosate application)? Presumably, since the transgenic EPSPS enzyme is insensitive to glyphosate, the shikimic acid pathway should not be compromised, and susceptibility to root diseases should not be affected. This has been confirmed in field studies with glyphosate-resistant soybeans and sudden death syndrome caused by *Fusarium solani* (Martius) Sacc., where glyphosate application did not increase disease.[47–49] Similar results were seen with *R. solani* AG-4 on glyphosate-resistant soybean[50] and glyphosate-resistant cotton[51,52] and with *Sclerotinia sclerotiorum* (Lib.) de Bary on glyphosate-resistant soybean.[53] However, there are some greenhouse and growth chamber experiments where glyphosate applications increased the disease levels on glyphosate-resistant soybean compared with unsprayed plants[49] and increased fungal colonization of soybean roots.[54,55] With sugar beet, more disease severity from *R. solani* and *Fusarium oxysporum* Schlecht. was detected in the greenhouse when glyphosate was applied to glyphosate-resistant cultivars, and shikimic acid accumulation was reduced compared with a susceptible cultivar.[56] The present results on wheat agree with the first set of studies, that glyphosate-sensitive wheat genotypes are as susceptible to root pathogens as glyphosate-resistant genotypes, both with and without glyphosate application.

Another possibility is that glyphosate could have a direct effect on the pathogenic fungi, since fungi also have the EPSPS enzyme, which is sensitive to the herbicide. However, in pure culture, most fungi were only inhibited at concentrations of 100–1000 mg g^{-1},[57] concentrations far higher than those that would be encountered in field soils. However, recent reports have shown that rusts on GR wheat and soybean could be inhibited by application of glyphosate.[58–60] Biotrophic fungi in the leaf tissues of GR plants may encounter higher concentrations of glyphosate than necrotrophic fungi in the roots or soil.

In contrast to the results with the pathogen and host crop alone, the presence of crop volunteer and changes in timing of glyphosate application had a profound effect on the disease and pathogen inoculum potential, and this effect was seen with both cultivars. In general, the longer the crop volunteer was left before spraying out, the greater was the competitive effect on the crop plant, and this was more profound with number of tillers and root length than with plant height. The number of tillers can be reduced by high plant density and competition,[61] whereas plants can respond to shading with increased plant height.

PRE glyphosate–weed management systems evaluated in this study were intended to mimic current best management practices for minimizing the inoculum potential of root-rotting pathogens before glyphosate-sensitive cereal crops are sown.[13] By spraying weeds out before planting, the inoculum carryover facilitated by a greenbridge can be minimized. Similarly, PRE + POST systems were intended to represent predicted best management practices for glyphosate-resistant wheat production. In contrast, the PLANTING and POST treatments represent worst-case scenarios, but it would still be possible to control weeds if the crop were glyphosate resistant. In these last two spray scenarios, the planted crop would be in contact with large crop volunteer plants dying from glyphosate application and colonized by root pathogens, an ideal situation for greenbridging to occur. The assay of *Rhizoctonia* in soil confirmed that the highest activity was seen in the PLANTING and POST treatments, suggesting that the roots of dying glyphosate-sensitive volunteer winter wheat plants in these two glyphosate–weed management systems provided a food base large enough to increase the activity of *R. solani*. Both *R. solani* and *G. graminis* var. *tritici* were favored owing to the greenbrige effect of crop volunteer with glyphosate application at the PRE and PRE + POST stage, compared with treatments without volunteer,

resulting in significant reductions in plant height, number of tillers and root length.

Why was this greenbridge effect seen only with *R. solani* and *G. graminis* var. *tritici* and not *P. ultimum* or *R. oryzae*? Differences in pathogen biology appeared to affect their pathogenesis on glyphosate-resistant wheat within glyphosate–weed management systems. *Pythium ultimum* commonly exists as sporangia in the soils and germinates in response to root exudates before colonizing glyphosate-sensitive plants.[62] Similarly, *R. oryzae* can convert from sclerotia to hyphae when stimulated by root exudates. Toothpick baiting did not detect *R. oryzae* activity for any glyphosate–weed management treatment, which supports the finding of Schroeder,[63] who reported that, 2 weeks after inoculum of *R. oryzae* was added to a natural soil, the number of colonies on toothpicks dramatically declined. The quiescent nature of *P. ultimum* and *R. oryzae* suggest that these pathogens will colonize glyphosate-treated glyphosate-sensitive cereal roots, but may have limited transmission to glyphosate-resistant wheat roots via the greenbridge effect. On the other hand, *G. graminis* var. *tritici* and *R. solani* can exist as active hyphal networks that search out novel energy sources, commonly the roots of neighboring plants.[12,13] The hyphal networks of *G. graminis* var. *tritici* and *R. solani* appeared to increase the zone of greenbridge influence by utilizing glyphosate-treated glyphosate-sensitive winter wheat as a food base. As the root mass of glyphosate-treated volunteer wheat increased, the levels of *R. solani* activity also increased. Increased *R. solani* activity due to volunteer crops using the toothpick baiting technique developed by Paulitz and Schroeder[41] has not been previously reported.

The response of wheat genotypes in *P. ultimum*-infested soils for PLANTING and POST glyphosate–weed management systems was unexpected. Plant tillering and root lengths of genotypes in the *P. ultimum*-infested PLANTING treatments were greater than those of glyphosate-resistant genotypes inoculated with the other pathogens or not inoculated. Also, plant height increased in *P. ultimum*-infested POST-EMERGENCE treatment. Root length and tillering are more sensitive to *Pythium* damage than height, and hence the effect showed up at earlier stages. A possible explanation for the consistent performance of glyphosate-resistant wheat in *P. ultimum*-infested soils is that this pathogen may have preferentially colonized the roots of glyphosate-sensitive volunteer winter wheat plants, causing stunting and thus reducing competition with glyphosate-resistant wheat genotypes. As primary colonizers, *Pythium* spp. have been reported to increase in the rhizosphere of GS plants as quickly as 2 h after the foliar application of glyphosate, and they colonize the roots within 2 days of glyphosate treatments.[43] This synergistic interaction between glyphosate and root colonization by *Pythium* spp. can increase the rate of death for glyphosate-sensitive plants.[9,10] In addition, the quantities of *P. ultimum* measured for all glyphosate–weed management systems far exceeded those predicted in natural soils. Levels of *P. ultimum* for glyphosate–weed management systems in the present experiments ranged from 3000 to 10 000 CFUs. Cook *et al*.[34] reported that levels of *Pythium* spp. measured in agricultural soils seldom exceed 500 CFUs. The high levels of *P. ultimum* may have aided in reducing competition as a result of this pathogen colonizing dying glyphosate-treated glyphosate-sensitive volunteer winter wheat. These high levels of *P. ultimum* did not result in deleterious effects on the plant health of glyphosate-resistant genotypes. The omnipresence of *Pythium* spp. in agricultural soils, and the density of glyphosate-sensitive weeds and volunteer plants, may determine the competitive effects on a glyphosate-resistant crop. This is the first published report suggesting that *Pythium* spp. preferentially colonize glyphosate-sensitive weeds to the benefit of a glyphosate-resistant crop.

These results indicate that PRE, PLANTING and POST systems increase the activity of *G. graminis* var. *tritici* and *R. solani*, and therefore the impact of the greenbridge effect must be addressed in glyphosate-resistant wheat systems. In order to minimize the damaging effects of these pathogens, producers may need to apply glyphosate at least 2–3 weeks before planting glyphosate-resistant wheat to decrease the inoculum potential of *G. graminis* var. *tritici* and *R. solani*, as currently advised for glyphosate-sensitive cereals. Techniques presented here may prove useful in the evaluation of other glyphosate-resistant crops species for the greenbridge effect. Field analyses varying the density, emergence and timing of glyphosate applications may provide additional information on the impact of glyphosate–weed management systems on disease pressure in glyphosate-resistant crops.

ACKNOWLEDGEMENTS

This work was supported by USDA Biotechnology Risk Assessment Grant 2001-03734 'Risk Assessment of Increased Soil-borne Disease Pressure in Glyphosate Tolerant Wheat Production', by USDA-ARS Project Number 5248-22000-012-00D and by Washington State University. The authors thank Vic DeMacon, Gary Shelton, Melissa McClendon, Julia Piaskowski, Crystal Hasey, Andrea Young, Jeannie Ellsworth, LaRena Claasen and Stephanie Swannick for their assistance. They also thank Monsanto and Westbred LLC for providing the glyphosate-resistant cultivars. References to a company and/or product by the USDA are only for the purposes of information and do not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

REFERENCES

1 Nail E, Young DL and Schillinger WF, Diesel and glyphosate price changes benefit the economics of conservation tillage versus traditional tillage. *Soil and Tillage Research* **94**:321–327 (2007).
2 Franz JE (ed.), *Glyphosate: a Unique Global Herbicide*, *ACS Monograph No. 189*. American Chemical Society, Washington, DC, 641 pp. (1997).
3 Herrmann K, The shikimate pathways as an entry to aromatic secondary metabolism. *Plant Physiol* **107**:7–12 (1995).
4 Steinrucken H and Amrhein N, The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl shikimic acid-3-phosphate synthase. *Biochem Biophys Res Comm* **94**:1207–1212 (1980).
5 Hammerschmidt R, Phytoalexins: what have we learned after 60 years? *Annu Rev Phytopath* **37**:285–306 (1999).
6 Moesta P and Griesbach H, L-a-aminooxy-b-phenylpropionic acid inhibits phytoalexin accumulation in soybean with concomitant loss of resistance against *Phytophthora megasperma* f. sp. *glycinea*. *Physiol Plant Pathol* **21**:65–70 (1982).
7 Sharon A, Amsellem Z and Gressel J, Glyphosate suppression of induced defense responses: increased susceptibility of *Cassia obtusifolia* to a mycoherbicide. *Plant Physiol* **98**:654–659 (1992).
8 Zhou H, Berg JD, Blank SE, Chay CA, Chen G, Eskelsen SR, *et al*, Field efficacy assessment of transgenic Roundup Ready wheat. *Crop Sci* **43**:1072–1075 (2003).
9 Lévesque CA and Rahe JE, Site, host specificity, and pathogenicity of *Pythium* spp. colonizers of glyphosate treated seedlings. *Can J Plant Pathol* **12**:336 (1990).
10 Lévesque CA and Rahe JE, Herbicide interactions with fungal root pathogens, with special reference to glyphosate. *Annu Rev Phytopathol* **30**:579–602 (1992).

11 Lévesque CA, Johal GS, Descalzo RC and Rahe JE, Participation of root rot fungi in the herbicidal activity of glyphosate. *Phytopathology* **90**:S99 (2000).
12 Hornby D, Bateman GL, Gutteridge RJ, Lucas P, Osbourn AE, Ward E, *et al*, Take-all and cereal production systems, in *Take-all Disease of Cereals*. CAB International, Cambridge, UK, pp. 103–164 (1998).
13 Smiley RW, Ogg A and Cook RJ, Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. *Plant Dis* **76**:937–942 (1992).
14 Roget DK, Neate SM and Rovira AD, Effect of sowing point design and tillage practice on the incidence of Rhizoctonia root rot, take-all and cereal cyst nematode in wheat and barley. *Aust J Exp Agric* **36**:683–693 (1996).
15 Weller DM, Cook RJ, MacNish G, Bassett EN, Powelson RL and Petersen RR, Rhizoctonia root rot of small grains favored by reduced tillage in the Pacific Northwest. *Plant Dis* **70**:70–73 (1986).
16 Pumphrey FV, Wilkins DE, Hane DC and Smiley RW, Influence of tillage and nitrogen fertilizer on Rhizoctonia root rot (bare patch) of winter wheat. *Plant Dis* **71**:125–127 (1987).
17 Smiley RW, Collins HP and Rasmussen PE, Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. *Plant Dis* **80**:813–820 (1996).
18 Smith JD, Kidwell KK, Evans MA, Cook RJ and Smiley RW, Assessment of spring wheat cultivars for disease reaction to *Rhizoctonia solani* AG-8 in controlled environment and direct-seeded field evaluations. *Crop Sci* **43**:694–700 (2003).
19 Smith JD, Kidwell KK, Evans MA, Cook RJ and Smiley RW, Evaluation of spring cereal grains and wild *Triticum* germplasm for resistance to *Rhizoctonia solani* AG-8. *Crop Sci* **43**:701–709 (2003).
20 Tinline RD, Bailey KL and Harding H, Role of plant breeding in controlling soil-borne diseases. *Can J Plant Pathol* **11**:158–165 (1989).
21 Rothrock CS, Relative susceptibility of small grains to take-all. *Plant Dis* **72**:883–886 (1988).
22 Higginbotham RW, Paulitz TC and Kidwell KK, Virulence of *Pythium* species isolated from wheat fields of eastern Washington. *Plant Dis* **88**:1021–1026 (2004).
23 Higginbotham RW, Paulitz TC and Kidwell KK, Evaluation of adapted wheat cultivars for tolerance to Pythium root rot. *Plant Dis* **88**:1027–1032 (2004).
24 Cook RJ and Veseth RJ, *Wheat Health Management*. American Phytopathological Society Press, St Paul, MN (1991).
25 Nida DL, Kolacz KH, Buehler RE, Deaton WR, Schuler WR, Armstrong TA, *et al*, Glyphosate-tolerant cotton: genetic characterization and protein expression. *J Agric Food Chem* **44**:1990–1996 (1996).
26 Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, *et al*, Development, identification, and characterization of a glyphosate-tolerant soybean line. *Crop Sci* **35**:1451–1461 (1995).
27 Sidhu RS, Hammond BG, Fuchs RL, Mutz J, Holden LR, George B, *et al*, Glyphosate-tolerant corn: the composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays* L.). *J Agric Food Chem* **48**:1967–1974 (2000).
28 Acreage. National Agricultural Statistics Service (2006).
29 Ogg AG and Isakson PJ, Agronomic benefits and concerns for Roundup-Ready® wheat. *Proc West Soc Weed Sci* **54**:80–90 (2001).
30 Halford N, Prospects for genetically modified crops. *Ann Appl Biol* **145**:17–24 (2004).
31 Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, *et al*, Engineering provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**:303–305 (2000).
32 Baley GJ, Disease response of glyphosate resistant wheat genotypes to soil-borne fungal pathogens. *PhD Thesis*, Washington State University, Pullman, WA (2005).
33 Mircetich SM and Kraft JM, Efficiency of various selective media in determining *Pythium* populations in soil. *Mycopathol Mycol Appl* **50**:151–161 (1973).
34 Cook RJ, Chamswarng C and Tang WH, Influence of wheat chaff and tillage on *Pythium* populations in soil and *Pythium* damage to wheat. *Soil Biol Biochem* **22**:939–947 (1990).
35 Cheng M, Fry JE, Pang SZ, Zhou HP, Hironaka CM, Duncan DR, *et al*, Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. *Plant Physiol* **115**:971–980 (1997).
36 Zadoks JC, Chang TT and Konzak CF, A decimal code for the growth stages of cereals. *Weed Res* **14**:415–421 (1974).
37 Anderson KA, Cobb WT and Loper BR, Analytical method for determination of shikimic acid: shikimic acid proportional to glyphosate application rates. *Comm Soil Sci Plant Anal* **32**:2831–2840 (2001).
38 Paulitz TC, Smith JD and Kidwell KK, Virulence of *Rhizoctonia oryzae* on wheat and barley cultivars from the Pacific Northwest. *Plant Dis* **87**:51–55 (2003).
39 Gomez KA and Gomez AA, Three-or-more factor experiments, in *Statistical Procedures for Agricultural Research*, 2nd edition. John Wiley & Sons, Inc., New York, NY, pp. 130–186 (1984).
40 Kramer CY, Extension of multiple range tests to group correlated adjusted means. *Biometrics* **13**:13–18 (1957).
41 Paulitz TC and Schroeder KL, A method for the quantification of *Rhizoctonia solani* and *Rhizoctonia oryzae* from soil. *Plant Dis* **89**:767–772 (2005).
42 Dissanayake N, Hoy J and Griffin J, Herbicide effects on sugarcane growth, Pythium root rot and *Pythium arrhenomanes*. *Phytopathology* **88**:530–535 (1998).
43 Lévesque CA, Rahe JE and Eaves DM, Fungal colonization of glyphosate-treated seedlings using a new root plating technique. *Mycol Res* **97**:299–306 (1993).
44 Descalzo R, Punja Z, Lévesque C and Rahe J, Identification of *Pythium* species as glyphosate synergists on bean (*Phaseolus vulgaris*) grown in different soils. *Mycol Res* **100**:971–978 (1996).
45 Smith DA and Hallett SB, Interactions between chemical herbicides and the candidate bioherbicide *Microsphaeropsis amaranthi*. *Weed Sci* **54**:532–537 (2006).
46 Boyette CD, Reddy KN and Hoagland RE, Glyphosate and bioherbicide interactions for controlling kudzu (*Pueraria lobata*), redvine (*Brunnichia ovata*), and trumpetcreeper (*Campsis radicans*). *Biocontrol Sci Technol* **16**:1067–1077 (2006).
47 Njiti VN, Myers O, Schroeder D and Lightfoot DA, Roundup ready soybean: glyphosate effects on *Fusarium solani* root colonization and sudden death syndrome. *Agron J* **95**:1140–1145 (2003).
48 Sanogo S, Yang XB and Lundeen P, Field response of glyphosate-tolerant soybean in herbicides and sudden death syndrome. *Plant Dis* **85**:773–779 (2001).
49 Sanogo S, Yang XB and Scherm H, Effects of herbicides on *Fusarium solani* f. sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. *Phytopathology* **90**:57–66 (2000).
50 Harikrishnana R and Yang XB, Effects of herbicides on root rot and damping-off caused by *Rhizoctonia solani* in glyphosate-tolerant soybean. *Plant Dis* **86**:1369–1373 (2002).
51 Baird R, Batson W, Watson C and Hightower P, Evaluation of transgenic cotton varieties and a glyphosate application on seedling disease incidence. *Mycopathologia* **158**:363–368 (2004).
52 Pankey JH, Griffin JL, Colyer PD, Schneider RW and Miller DK, Preemergence herbicide and glyphosate effects on seedling diseases in glyphosate-resistant cotton. *Weed Technol* **19**:312–318 (2005).
53 Lee CD, Penner D and Hammerschmidt R, Influence of formulated glyphosate and activator adjuvants on *Sclerotinia sclerotiorum* in glyphosate-resistant and -susceptible *Glycine max*. *Weed Sci* **48**:710–715 (2000).
54 Kremer RJ, Means NE and Kim S, Glyphosate affects soybean root exudation and rhizosphere micro-organisms. *Internat J Environ Anal Chem* **85**:1165–1174 (2005).
55 Means NE and Kremer RJ, Influence of soil moisture on root colonization of glyphosate-treated soybean by *Fusarium* species. *Comm Soil Sci Plant Anal* **38**:1713–1720 (2007).
56 Larson RL, Hill AL, Fenwick A, Kniss AR, Hanson LE and Miller SD, Influence of glyphosate on Rhizoctonia and Fusarium root rot in sugar beet. *Pest Manag Sci* **62**:1182–1192 (2006).
57 Franz JE, Mao MK and Sikorski JA, Toxicology and environmental properties of glyphosate, in *Glyphosate: a Unique Global Herbicide*, *ACS Monograph No. 189*, ed. by Franz JE. American Chemical Society, Washington, DC, pp. 103–142 (1997).
58 Feng PCC, Baley GJ, Clinton WP, Bunkers GJ, Alibhai MF, Paulitz TC, *et al*, Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean. *Proc Natl Acad Sci USA* **102**:17 290–17 295 (2005).
59 Anderson JA and Kolmer JA, Rust control in glyphosate tolerant wheat following application of the herbicide glyphosate. *Plant Dis* **89**:1136–1142 (2005).
60 Feng PCC, Clark C, Andrade CG, Balbi MC and Caldwell C, The control of Asian rust by glyphosate in glyphosate-resistant soybean. *Pest Manag Sci* **64**:353–359 (2008).

61 Carr PM, Horsley RD and Poland WW, Tillage and seeding rate effects on wheat cultivars: II. Yield components. *Crop Sci* **43**:210–218 (2003).
62 Stanghellini ME, Spore germination, growth, and survival of *Pythium* in soil. *Proc Am Phytopathol Soc* **1**:211–214 (1974).
63 Schroeder KL, The dynamics of root diseases of wheat and barley in the transition from conventional tillage to direct seeding. *Dissertation*, Washington State University, Pullman, WA (2004).